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SYNTHESIS OF AN ^{125}I ANALOG OF MK-0591 AND CHARACTERIZATION OF A 5-LIPOXYGENASE ACTIVATING PROTEIN BINDING ASSAY

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Summary

The ^{125}I analog of MK-0591,**1**, has been prepared for use as a radioligand for developing a 5-lipoxygenase activating protein (FLAP) binding assay. The radiosynthesis involves a two step oxidative iododestannylation-saponification procedure. A FLAP binding assay has been developed in human neutrophil membranes. The binding of **1** to human neutrophil FLAP is rapid, reversible, of high affinity, saturable and selective for FLAP inhibitors.

Keywords: radioligand, iodine-125, 5-lipoxygenase activating protein (FLAP), FLAP binding assay.

Introduction

Prasit *et al* have recently described a new class of leukotriene biosynthesis inhibitor exemplified by MK-886 and MK-0591(1). This class of inhibitor does not inhibit the 5-lipoxygenase (5-LO) enzyme by direct interaction but rather by binding to 5-lipoxygenase activating protein (FLAP) in the cell membrane to prevent activation of 5-LO (2). Since both 5-LO and FLAP are essential for cellular synthesis of leukotrienes, compounds which interact with either of these proteins are potentially useful in a number of inflammatory and hypersensitivity disorders including asthma, psoriasis and allergic rhinitis where leukotrienes are implicated in the pathophysiology (3). We required a radioligand suitable for developing a binding assay to measure the affinity of compounds for FLAP. We now describe the synthesis of the ^{125}I analog of MK-0591,**1** and characterization of its binding to human neutrophil membranes.

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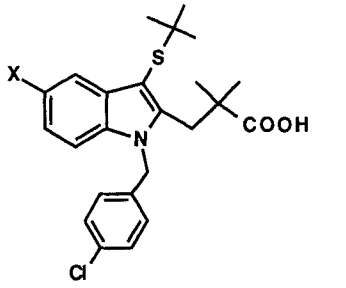
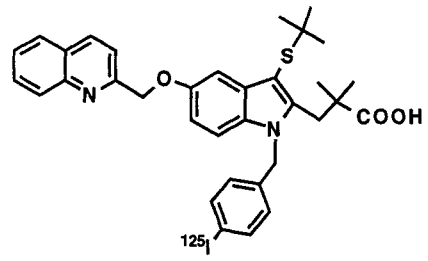


MK-886 X=$(CH_3)_2CH$-

MK-0591 X=



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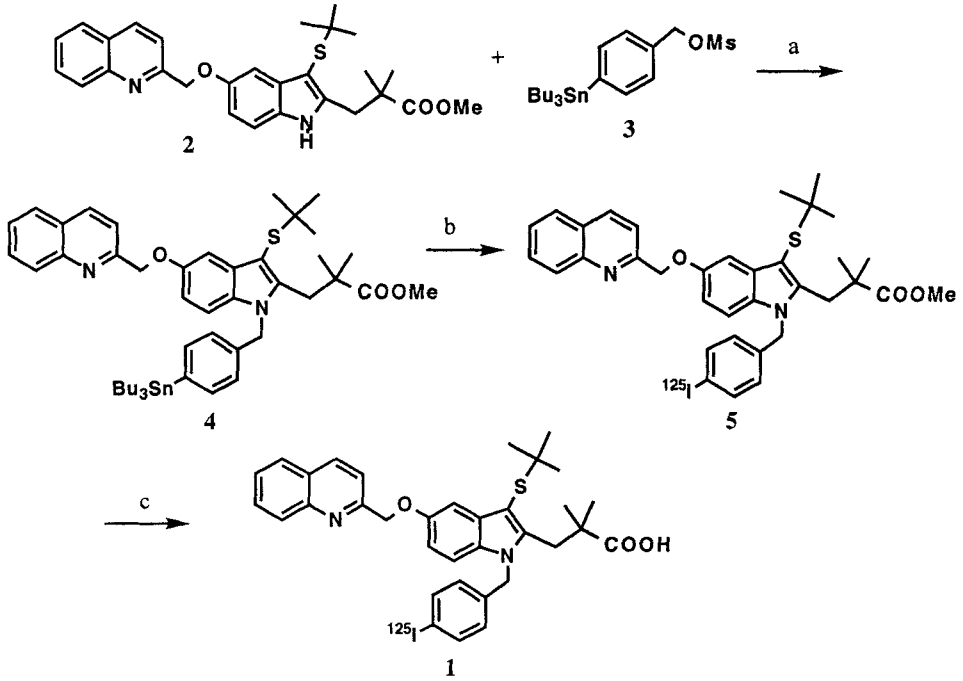
Results and Discussion

(I) Synthesis of the Radioligand

The synthetic route used for the preparation of the ^{125}I analog of MK-0591, **1**, is shown in Scheme 1. 3-[3-tert-Butylsulfanyl-5-(quinolin-2-ylmethoxy)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **2** was prepared in eight steps as described in the literature (4). Alkylation of **2** with methanesulfonic acid

SCHEME 1

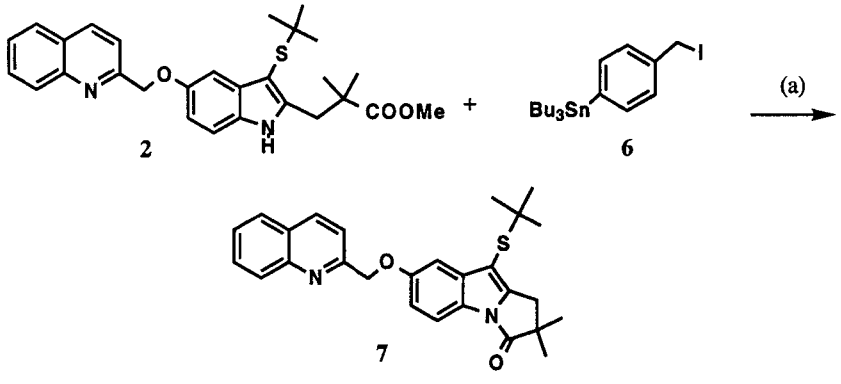


(a) NaH/DMF (b) Na ^{125}I, chloramine-T, DMF (c) NaOH/MeOH, citric acid

4-tributylstannanyl-benzyl ester **3** using sodium hydride in dimethylformamide afforded 3-[3-tert-butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-tributylstannyl-benzyl)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **4** in good yield. Oxidative iododestannylation of **4** using Na ^{125}I and chloramine-T in dimethylformamide gave 3-[3-tert-butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-[^{125}iodo]-benzyl)1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **5**, which upon saponification gave the ^{125}I analog of MK-0591, **1**. Non-labelled **1** was prepared by alkylation of **2** with 4-iodo-benzyl bromide to afford non-labelled ester, **5**, which was saponified to the corresponding acid (see Experimental).

The use of methanesulfonic acid 4-tributylstannyl-benzyl ester **3** as the alkylating agent was crucial to the success of step (a) in Scheme 1. For example, attempts to alkylate **2** under a variety of conditions, using less reactive alkylating agents such as 4-tributylstannyl-benzyl iodide **6** did not give **5**, but rather 8-tert-butylsulfanyl-2,2-dimethyl-6-(quinolin-2-ylmethoxy)-1,2-dihydro-3a-aza-cyclopenta [a] inden-3-one **7**, the result of intramolecular acylation of **2** (Scheme 2).

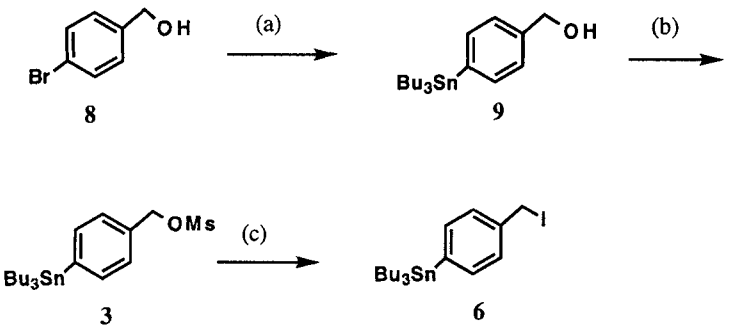
SCHEME 2



a) K_2CO_3/DMF or NaH/DMF

There are no reports in the literature of syntheses of tributylstannyl-benzyl esters of methanesulfonic acid. Perhaps this is a reflection of the reputed high reactivity and instability of methanesulfonic acid benzyl esters in general (5). In any event, the methanesulfonic acid 4-tributylstannyl-benzyl ester **3** was easily prepared as shown in Scheme 3. 4-Bromo-benzyl alcohol **8** was treated with two equivalents of n-butyllithium at -70°C to affect metal-halogen exchange, then alkylated with tri-n-butyltin chloride to give 4-tributylstannyl-benzyl alcohol **9**. Treatment of **9** with methanesulfonyl chloride and triethylamine in dichloromethane gave methanesulfonic acid-4-tributylstannyl-benzyl ester **3** in excellent yield. The mesylate **3** was converted to the corresponding iodide **6** by simply stirring with sodium iodide in THF.

SCHEME 3



(a) n-BuLi, n-Bu_3SnCl, -78°; (b) MsCl, Et_3N, CH_2Cl_2 (c) NaI, THF.

(II) Development of a Binding Assay.

We have utilized radioligand binding techniques with **1** (specific activity = 2200 Ci/mmole) to examine FLAP binding sites in purified human neutrophils. Neutrophils were isolated from 100 mL blood of healthy donors using a modified Fiscoll Hypaque method. Isolated neutrophils were suspended in 50 mM K_3PO_4, 0.1 M NaCl, 2 mM EDTA, 1 mM DTT, 0.5 mM PMSF and 60 μg/mL soybean trypsin inhibitor (pH=7.3). The cell suspension was sonicated with a Branson microprobe, 3x20 sec at 4°C and the homogenate was centrifuged at 10,000 xg for 15 min at 4°C. The resulting supernatant was then centrifuged at 100,000 xg for 60 min at 4°C. The pellet was resuspended at a protein concentration of 3-5 mg/mL and stored at -25°C.

Binding assays with **1** were routinely performed in a 210 μL volume containing membrane preparations (0.3 - 10 μg/mL), 100 mM Tris • HCl (pH=7.5), 140 mM NaCl, 2 mM EDTA, 0.5 mM DTT, 5% glycerol, 0.05% Tween-20, the radioligand (0.05 -7 nM) and with or without 2 μL of competitor. The reaction mixture was incubated at 21°C for 20 min. At the end of incubation, 3 mL of cold wash buffer (100 mM Tris • HCl plus 0.05% Tween-20) were added to assay tubes, and samples were immediately filtered through Whatman GF/C glass fiber filters to separate free and bound **1**. Filters were rapidly washed with 3x3 mL of the wash buffer, dried and counted in a gamma radioactivity counter. Specific binding, defined as binding in the absence of non-labelled **1** minus binding in the presence of 1 μM non-labelled **1**, was >80% of total binding in human neutrophil membranes.

The radioligand **1** bound rapidly to neutrophil membranes. Equilibrium was reached within 2-10 min at 21°C and **1** was rapidly dissociated from the binding sites with a half-time ($t_{1/2}$) of approximately 2 min in the presence of an excess of non-labelled **1** (1 μM). Figure 1 shows linearity of radioligand binding with increasing concentrations of neutrophil membrane proteins. Both total and specific binding of **1** increased with protein amounts from 0.325 to 4 μg. Thereafter, ligand binding increased slightly.

Figure 1. Binding of 1 to Human Neutrophil Membranes as a Function of Protein Amount

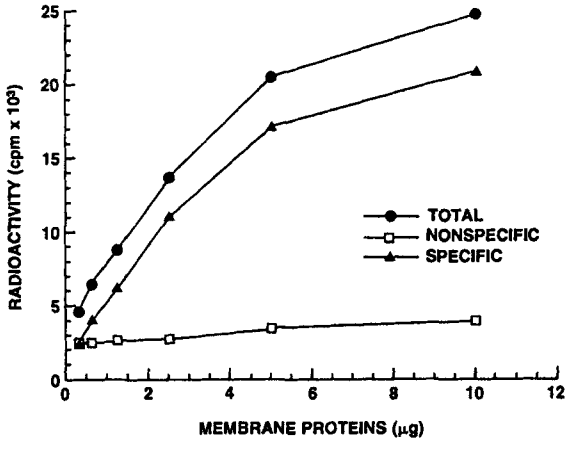
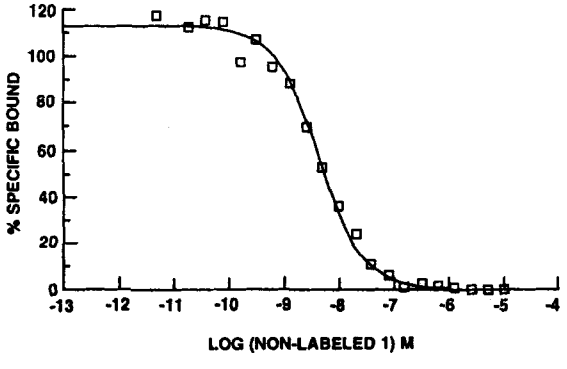


Figure 2 shows that non-labelled **1** effectively competed with **1** for neutrophil membrane binding sites with an IC_{50} = 3.4 nM. Non-linear least-squares analysis of data of the experiments reveals that the curve is best described by the competition of non-labelled **1** with **1** for a single homogeneous population of binding sites [i.e., one-site (two parameter) fit model] (6), giving estimated *Ki* and receptor concentration of 2.9 ±

Figure 2. Competition of Non-labelled 1 Binding to Human Neutrophil Membranes



2.4 nM (mean ± SE) and 63 ± 49 pmol/mg protein, respectively. Scatchard analysis of the result of equilibrium binding experiments confirms the presence of a single population of binding sites with a Kd = 2.2 nM and a Bmax =19 pmol/mg protein.

Table 1 illustrates the result of competition experiments in human neutrophil membranes. Known FLAP inhibitors, MK-0591 and BAY-X1005 (7), inhibited binding of **1** with the IC_{50} values similar to those reported previously in the literature (8, 9). Arachidonic acid, LTD_4, LTB_4, 5-LO inhibitors (10, 11) and a LTD_4 antagonist (12) had little or no effect. Thus, the binding of **1** is rapid, reversible, of high affinity, saturable, and selective for FLAP inhibitors, demonstrating the fulfillment of pharmacological criteria of its binding to FLAP binding sites in human neutrophil membranes.

Table 1. Selectivity of Competition with **1** Binding to Human Neutrophil Membranes

Competitor	IC_{50}, μM
Arachidonic acid	290
LTB_4	>10
LTD_4	>10
FLAP Inhibitors	
Non-labelled **1**	0.003
BAY-X1005	0.19
MK-0591	0.003
5-LO Inhibitors	
Zilueton	>100
D-2138	>100
LTD_4 Antagonist	
ICI-204,219	>100

Experimental

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were obtained on Bruker (AM 300) or Varian (XL250 or T69) instruments. Mass spectra were run on a Hewlett Packard particle beam mass spectrometer.

3-[3-tert-Butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-tributylstannyl-benzyl)-1H-indol-2-yl]-2,2-dimethyl propionic acid methyl ester **4**. A solution of 300 mg (.63 mmole) of 3-[3-tert-Butylsulfanyl-5-(quinolin-2-yl methoxy)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **2** (4) in 10 mL of DMF was cooled to 0°

in a ice-water bath. Sodium hydride [60% dispersion in mineral oil, 33 mg (.81 mmole)] was added in one portion and mixture stirred at 0° for 5 min. A solution of 578 mg (1.2 mmole) of methanesulfonic acid 4-tributylstannanyl-benzyl ester **3** in 1 mL of DMF was added and the mixture was stirred at 0° for 15 min. The reaction was diluted with H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography on silical gel eluting with IPE/hexanes to give 200 mg (36%) of **4** as an oil • TLC: IPE/hexanes::20/80, single-spot material R_f 0.5 • ^{1}H-NMR (300 MHz, $CDCl_3$) δ: (0.88, t, 9H), (1.00, t, 6H), (1.15, s, 9H), (1.20, s, 6H), (1.29, m, 6H), (1.55, m, 6H), (3.31, s, 2H), (3.60, s, 3H), (5.35, s, 2H), (5.95, s, 2H), (6.77, d, 2H), (6.92, d, 1H), (7.09, d, 1H) (7.32, d, 2H), (7.40, d, 1H), (7.53, t, 1H), (7.75, m, 3H) (8.14, dd, 2H).

3-[3-tert-Butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-[^{125}iodo]-benzyl)1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **5**. In a "V" vial was dispensed 10.5 mCi (25 μL, 4.5 nmole) of sodium iodide [^{125}I], NEZ-033H, in 0.1N NaOH. To this was added 50 μg (58 nmole) of 3-[3-tert-butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-tributylstannyl-benzyl)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **4** dissolved in 50 μL DMF. Reaction was initiated by the addition of 20 μg (71 nmole) of Chloramine-T dissolved in 10 μL of DMF and allowed to proceed for 20 minutes. After this time, 100 μL of HPLC eluant was added to obtain a reasonably quantitative transfer and the material was purified. Purification was done in HPLC using a Waters uBondapak C-18 column (3.9 x 300 mm) using 85:15:0.1 methanol/water/acetic acid as eluant. Waters Model 501 pumps were used along with a Waters Automated Gradient Controller. UV detection was done using a Applied Biosystems Model 757 Absorbance Detector set at 254 nm. Radiochemical detection was done using a Nico GM tube placed in-line to the elution tube. The material was injected and the radioiodinated ester eluted at 16 minutes yielding 4.4 mCi. The resulting pool of **5** was dried down *in vacuo* to carry on to the next step.

3-[3-tert-Butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-[^{125}iodo]-benzyl-1H-indol-2-yl]-2,2-dimethyl-propionic acid **1**. The ester **5** was saponified to **1** by addition of 150 μL of methanol to the dried-down pool followed by 50 μL of water and 50 μL of 1**N** NaOH. The reaction was allowed to proceed overnight at ambient temperature. The reaction was then acidified with 100 μL of 0.5 **M** citric acid and the resulting mixture was repurified in the above HPLC system. The acid **1** eluted at 13 minutes yielding 2.5 mCi, which corresponds to a specific activity of approximately 2200 Ci/mmole. The material was checked for peak singularity using a TLC system of 95:5 chloroform/methanol on silica gel (R_f 0.25). Identity confirmed by comparison with non-labeled **1**.

8-tert-Butylsulfanyl-2,2-dimethyl-6-(quinolin-2-ylmethoxy)-1,2-dihydro-3a-aza-cyclopenta [a] inden-3-one **7**. A mixture of 160 mg (0.33 mmoles) of 3-[3-tert-butylsulfanyl-5-(quinolin-2-ylmethoxy)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **2**, 309 mg (.67 mmole) of methanesulfonic acid 4-iodobenzyl benzyl ester **6** and 138 mg (1 mmole) of K_2CO_3 in 5 mL of DMF was stirred at ambient temperature for 60 hrs. The reaction was diluted with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and volatiles were evaporated. The residue was purified by column chromatography on silica gel using CH_2Cl_2 as eluant to give 130 mg (58%) of **7**; mp 134-135°C • TLC: CH_2Cl_2, single-spot material R*f* 0.20 • ^{1}H-NMR (300 MHz $CDCl_3$) δ: (1.19, s, 9H), (1.42, s, 6H), (3.05, 5, 2H) (5.46, s, 2H), (7.08, d, 1H), (7.28, s,1H), (7.55, t, 1H), (7.70, m, 3H), (7.95, d, 1H), (8.15, dd, 2H).

4-tributylstannyl-benzyl alcohol **9**. A solution of 2g (10.6 mmole) 4-bromobenzyl alcohol **8** in 50 mL of THF was cooled to -70°. n-BuLi [1.6 **M** in hexane, 13.7 mL, (22 mmole)] was added dropwise. The mixture was stirred at -70° for 90 minutes. A solution of 7.15 g (22 mmole) of tri-n-butyltin chloride in 5 mL of THF was added dropwise at -70° and the reaction was allowed to warm to room temperature. The reaction was quenched by addition of 5 mL of a saturated aqueous ammonium chloride solution. The mixture was extracted with EtOAc and the organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with hexanes/CH_2Cl_2 to give 1.4g of **9** (33%) as an oil • TLC:CH_2Cl_2, single-spot material R*f* 0.40 • ^{1}H-NMR (300 MHz $CDCl_3$) δ: (0.90, t, 9H), (1.08, t, 6H), (1.30, m, 6H), (1.60, m, 6H), (4.66, d, 2H) (7.30, d, 2H), (7.50, d, 2H) • Mass Spectrum, m/e 398 (parent +1), 308, (base).

Methanesulfonic acid-4-tributylstannyl-benzyl ester **3**. A solution of 397 mg (1 mmole) of 4-tributylstannyl-benzyl alcohol **9** and 151 mg (1.5 mmole) TEA in 20 mL CH_2Cl_2 was cooled to 0°C. A solution of 125 mg (1 mmole) of methanesulfonyl chloride in 2 mL CH_2Cl_2 was added dropwise. After 30 minutes at 0° the reaction was diluted with water and the organic layer was separated and washed with 1N HCl. The organic layer was dried over Na_2SO_4 and evaporated to afford 320 mg of **3** (67%) as an oil which was used without purification • TLC CH_2Cl_2 single-spot material R*f* 0.70 • ^{1}H-NMR (300 MHz $CDCl_3$) δ: (0.90, t, 9H), (1.90, t, 6H), (1.33, m, 6H), (1.55, m, 6H), (2.90, s, 3H), (5.24, s, 2H), (7.48, d, 2H), (7.50, d, 2H).

4-tributylstannyl-benzyl iodide **6**. Sodium iodide 450 mg (3 mmole) was added to a solution of 1g (2 mmole) of methanesulfonic acid-4-tributylstannyl-benzyl ester **3** in 10 mL of THF. The reaction was

stirred at ambient temperature for 15 minutes. Water was added and the organic layer was separated and dried over Na_2SO_4. The solvent was evaporated to give 1g of **6** (100%) as an oil • TLC CH_2Cl_2 single-spot material R*f* 0.85 • ^{1}H-NMR (300 MHz $CDCl_3$) δ: (0.90, t, 9H), (1.91, t, 6H), (1.35, m, 6H), (1.54, m, 6H), (4.45, s, 2H), (7, 40, m, 4H).

3-[3-tert-Butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-iodo-benzyl)-1H-indol-2-gl]-2,2-dimethyl-propionic acid methyl ester **5** (non-labelled). A mixture of 144 mg (.3 mmole) of 3-[3-tert-butylsulfanyl-5-(quinolin-2-ylmethoxy)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **2**, 180 mg (.6 mmole) of 4-iodo-benzyl-bromide and 125 mg (.9 mmole) of potassium carbonate in 2 mL DMF was stirred at ambient temperature for 48 hrs. The reaction was diluted with EtOAc and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel eluting with IPE/hexanes::1/1. After elution of the less polar tricyclic **7**, 100 mg of non-labelled **5** (43%) was collected, mp-164-166°C • TLC IPE/hexanes:: 50/50, single-spot material R*f* 0.25 • ^{1}H-NMR (300 MH_7, $CDCl_3$) δ:(1.14, s, 9H), (1.20, s, 6H), (3.25, s, 2H), (3.60, s, 3H), (5.30, s, 2H), (5.45, s, 2H), (6.55, d, 2H), (6.95, m, 2H), (7.43, d, 1H), (7.55, d, 3H), (7.75, m, 3H), (8.10, dd, 2H).

3-[3-tert-Butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-iodo-benzyl)-1H-indol-2-yl]-2,2-dimethyl-propionic acid **1** (non-labelled). A solution of 80 mg (.11 mmole) of 3-[-3-tert-butylsulfanyl-5-(quinolin-2-ylmethoxy)-1-(4-iodo-benzyl)-1H-indol-2-yl]-2,2-dimethyl-propionic acid methyl ester **5** (non-labelled) in 5 mL of THF and 5 mL of MeOH containing .11 mL (.55 mmoles) 5 **N** NaOH was stirred at ambient temperature for 24 hrs. The reaction was diluted with water, acidified to pH 5 with 2 **N** HCl and extracted with EtOAc. The organic layer was dried and evaporated. The residue was triturated with ether and filtered to give 50 mg of **1** (66%) (non-labeled), mp 172-174°C • TLC CH_2Cl_2/MeOH:: 9/1, single-spot material R*f* 0.10 • ^{1}H-NMR (300 MHz, $CDCl_3$), δ: (1.13, s. 9H), (1.25, s, 6H), (3.25, s, 2H), (5.35, s, 2H), (5.40, s, 2H), (6.50, d, 2H), (6.95, m, 2H), (7.42, d, 1H), (7.55, m, 3H), (7.75, m, 3H), (8.12, dd, 2H).

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